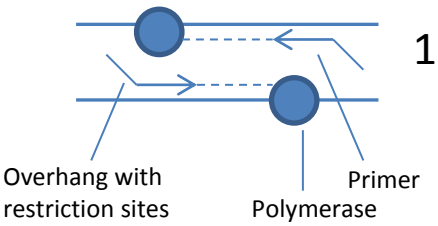
Cloning

The name comes from the fact that in the end one bacterial colony is testet, which derives from one single bacterium through cell division, also called clone. It has nothing to do with Dolly!

Generating new parts

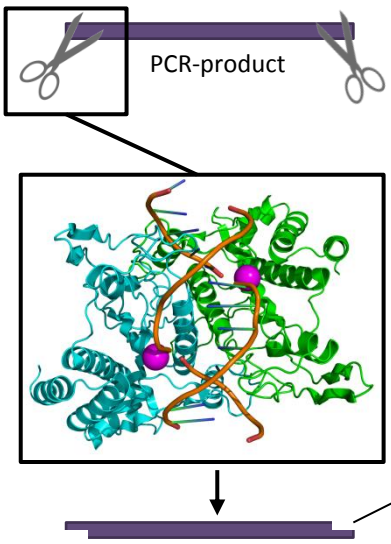


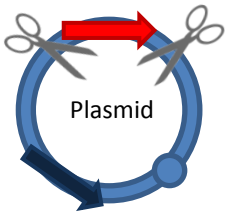
1. PCR

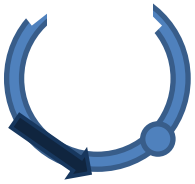
The amplification of spezific DNA parts (defined through the primers) with the help of a polymerase

Cloning two parts together

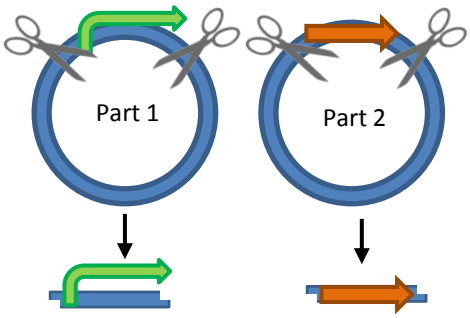
2. Restriction digest





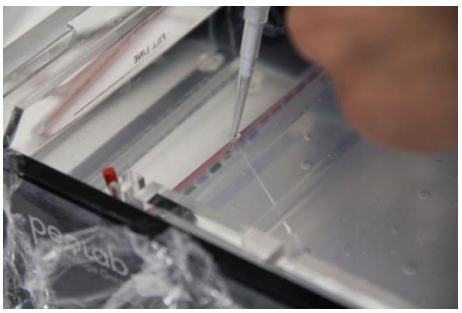


„sticky ends“

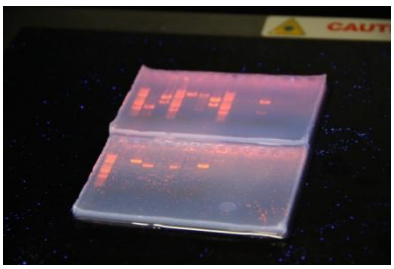


With the help of molecular scissors, so-called restriction enzymes,the DNA is cut and „sticky ends“ are generated. These stick to other DNA-parts with the same „sticky ends“

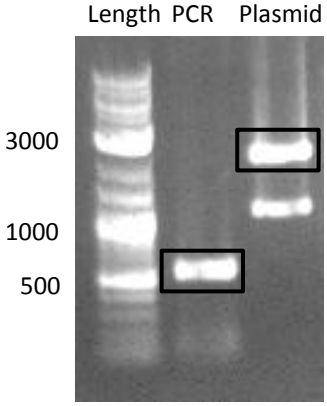
3. Gel electrophoresis



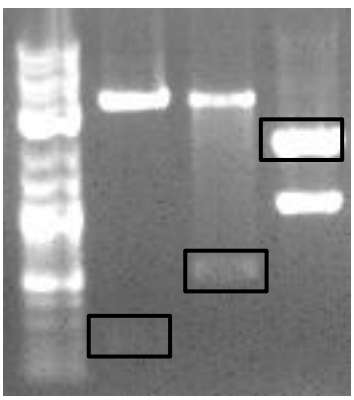
DNA is negatively charged and migrates in a gel mesh.towards the plus pol if charge is applied. As longer parts have a harder time getting through the mesh as smaller parts, a fractionation of the DNA-parts after the size occurs. The DNA is then visualized with a dye that fluoresces under UV-light



Length PCR Plasmid

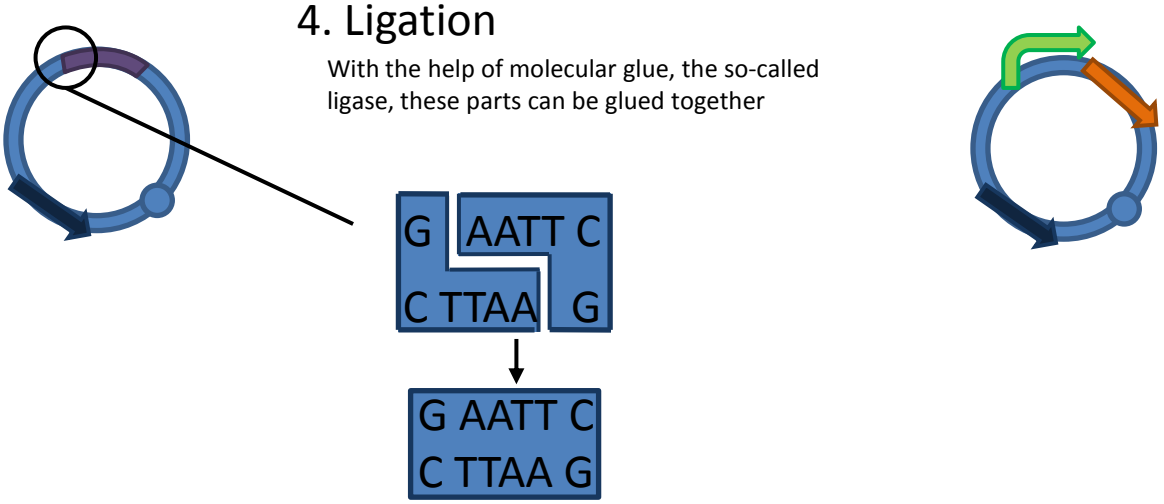


Part 1 Part 2 Plasmid



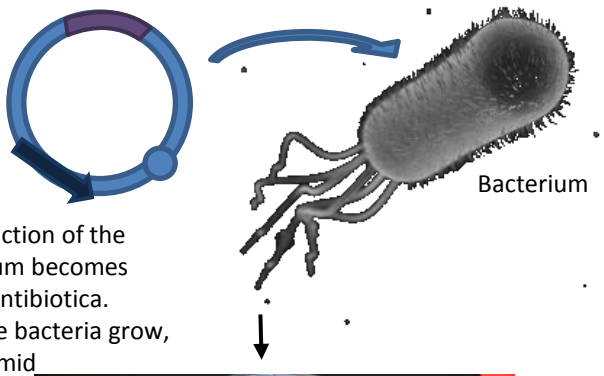
4. Ligation

With the help of molecular glue, the so-called ligase, these parts can be glued together

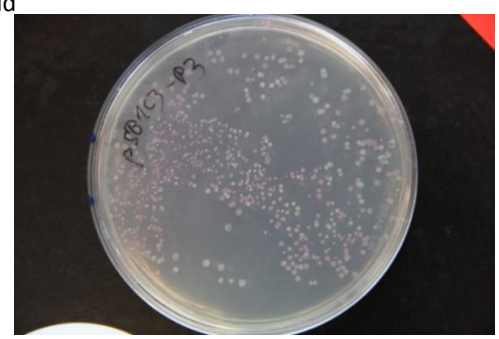


5. Transformation

One now introduces the plasmid into a bacterium by yielding the envolope „porous“ with help of heat and salt



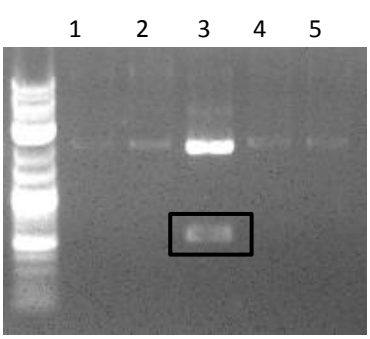
Through the introduction of the plasmid the bacterium becomes resistant againt an antibiotica. Therefore only those bacteria grow, which have the plasmid



The plasmid contains a gene as negative-marker which leads to red colonies. These are wrong.

6. Sreening

One innoculates different colonies in a growth media, extracts the plasmid-DNA and performs a test restroction digest to find the right plasmids



The plasmid from the right clone can then be sequenzed to verify the sequence.